Hittite Journal of Science and Engineering, 2014, (1) 1 37-41
ISSN NUMBER: 2149-2123
DOI: 10.17350/HJSE19030000006

Antioxidant and Antimicrobial Activities with GC/MS Analysis of the *Morus alba* L. Leaves

Asiye Aslı Emniyet[1], Emre Avcı[2], Burcin Ozcelik[1,*], Gulcin Alp Avcı[2] and Dursun Ali Kose[3]
[1] Hitit University, Department of Biology, Corum, TURKEY
[2] Hitit University, Department of Molecular Biology and Genetics, Corum, TURKEY
[3] Hitit University, Department of Chemistry, Corum, TURKEY

ABSTRACT

Morus alba L. (Moraceae) leaves are used effectively to treat fever, protect liver from damage, strengthen the joints, facilitate discharge of urine in Turkey folk medicine. In this study we aimed to determine in vitro antioxidant and antimicrobial activity with GC/MS analysis of the *Morus alba* L. leaves. Dried plant leaves samples are milled and extracted with distilled water and ethanol in Soxhlet machine. After extraction, extract samples were concentrated in rotary evaporator machine. Total antioxidant status values were determined as mmol Trolox Equivalent/L spectrophotometrically by using Erel's method. Sterile extracts were used to avoid contamination in antimicrobial activity test. As test microorganisms *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeroginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) were used. Antimicrobial activity was determined with disc diffusion method. We used ethanol extracts of samples in GC/MS analysis. Ethanol and distilled water extracts showed antioxidant activity in different rate. Besides, ethanol extracts have an antimicrobial activity but water extracts have not antimicrobial activity on our test microorganisms. We determined four compounds (9,12,15-octadecatrienoic acid, ethyl ester, linolenic acid ethyl ester, gibberellic acid) with GC/MS analysis in ethanol extracts.

In this research we enlightened antimicrobial and antioxidant activities with GC/MS analysis of *Morus alba* L. leaves. So this research supports of using this plant leaves in pharmacological and medical processes.

Key Words:
Antioxidant activity; Antimicrobial activity; GC/MS analysis; *Morus alba* L.; Extraction

Article History:
Received: 2014/11/11
Accepted: 2014/12/29
Online: 2014/12/31

Correspondence to: *Burcin Ozcelik, Hitit University, Faculty of Arts and Science, Department of Biology, Corum, Turkey*
Tel: +90 (364) 227 70 00 (1715)
Fax: +90 (364) 227 70 05
E-Mail: burcinozclk@gmail.com.tr
burcinozcelik@hitit.edu.tr

INTRODUCTION

Morus alba L. belongs to the Moraceae family, is widely cultivated and naturalized elsewhere and is one of the most important medicinal plant [1]. The mulberry (*Morus alba* L.) fruit is widely regarded as a nutritious food. Root, stem barks, twigs and leaves of mulberry have long been used in traditional medicine to treat fever, inflammation, hepatitis, cancer, diabetes, dislipidemia, diarrhoea, dyspepsia, hypertension, anthelmintic and to protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine, and lower blood pressure. Different parts of the mulberry have been extensively investigated for their various health benefits, including antioxidative, hypolipidemic and antiatherogenic effects [2-4].

Besides, this genus is known to be rich in flavonoids [5].

Reactive oxygen species (ROS) are produced in metabolic and physiological processes, and causes different chronic diseases like cancer and heart diseases [6]. Antioxidant molecules prevent such these harmful reactions [7]. All biological systems, including human beings, have antioxidant defence mechanisms. Since these natural antioxidant mechanisms can be inefficient, dietary intake of antioxidant compounds is important [8]. Mulberry leaf extract has been demonstrated to contain several substances that can act as potent antioxidants or free radical scavengers such as flavonoids and moracins [9].

Many plants contain a diverse array of compounds such as phenolic acids, flavonoids, tannins, vitamins, and terpenoids that account for their biological properties and the antioxidant and antimicrobial abilities [10,11]. In addition, plant and plant products can be used for isolating health-promoting bioactive compounds that have antioxidant, hypoglycemic, hypotensive and hypocholesterolemic effects [12]. For a few years, many researchers have intensively investigated antimicrobial properties of plant extracts and natural products as the demand for safe and new pharmaceuticals which has increased due to the misuse of antibiotics and an increase in immuno-deficiency [13].

In the present study, we aimed 1) to examine the antioxidant capacities of distilled water and ethanol extracts of *Morus alba* L. leaves, 2) to measure the antimicrobial activity of leaf extracts against five test microorganisms, 3) to determine main components of extracts with GC/MS analysis method.

MATERIALS AND METHODS

Plant Sample Collection

Morus alba L. leaves were collected from natural areas. Samples were identified by plant taxonomist Prof. Dr. Murat Ekici of the Department of Biology, Gazi University, Ankara. All plant samples were dried at room temperature in a few days.

Preparation of Extracts

Dry leaves were milled. To prepare extract two different solvent were used, ethanol and distilled water respectively, and 10 g sample weigh for each extraction. Extraction was made with soxhlet machine during 8 hours. After from extraction process, solvents were concentrated in rotary evaporator machine. Sterile extracts were used to avoid contamination in antimicrobial activity test. For this reason, extracts were filtered with micro filter which has 0.45 micrometer pore diameter. Extracts were protected from light and kept in +4°C until executing the experiments.

Determination of Total Antioxidant Status (TAS)

The total antioxidant status (TAS) was determined using a TAS Assay Kit (Rel Assay Diagnostics®, Gaziantep, Turkey) according to a novel automated measurement method developed by Erel in 2004 [7]. In this method, a hydroxyl radical was produced by the Fenton reaction and reacted with the colourless substrate o-dianisidine to produce the bright yellowish-brown dianisyl radical. For this procedure, 500 µL of reagent 1 was placed in the cell, and 30 µL of sample (standard) was added. The initial absorbance at 660 nm for the first absorbance point was measured in a spectrophotometer (Shimadzu UV mini-1240, Kyoto, Japan). Subsequently, 75 µL of reagent 2 was added to the cell and incubated for 10 min at room temperature. The absorbance at 660 nm was read for a second time. The results obtained from this process were calculated with the following formulas:

Results = [(ΔAbs Std 1)–(ΔAbs Sample)]/ [(ΔAbs Std 1)–(ΔAbs Std2)]

ΔAbsorbance Standard 1= (Second Absorbance of Std 1-First Absorbance of Std 1)

ΔAbsorbance Standard 2= (Second Absorbance of Std 2-First Absorbance of Std 2)

Δ Sample Absorbance = (Second Absorbance of Sample-First Absorbance of Sample)

The results were expressed as milimolar Trolox equivalents per liter (mmol Trolox Eq/L).

Determination of Antimicrobial Activity

As test microorganisms *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeroginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) were used. All strains were obtained from culture collection at Hitit University, Faculty of Science and Arts, Department of Molecular Biology and Genetic, Microbiology Research Laboratory Culture Collection.

The antimicrobial activities were evaluated by disc-diffusion method. Microorganisms were activated two times in nutrient broth and incubated for 16-24 hours at 37°C temperature. After activation of cultures, optical density (OD) arranged spectrophotometrically in 600 nanometer wavelength. Activated ($OD_{600} \approx 600$) microorganisms were inoculated nutrient agar and were spread all agar surface with drigalski bar. Sterile discs which were prepared from Whatman Filter paper were put and extracts were added to these discs in different volume, 10 and 20 microliter respectively. These petri dishes were incubated for 16-24 hours at 37°C temperature. When incubation time completed, all petri dishes were investigated. All antimicrobial activity zones were measured with ruler.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Extracts

The chemical compositions of the plant extracts were analysed by using GC–MS technique and the fragmentation analysis was performed. The mass spectrometer was Thermo Scientific DSQ II Single Quadrupole GC/MS in the electron impact (EI)

ionization mode (70 eV) and HP- 5MS (bonded and cross-linked 5% phenyl-methylpolysiloxane, 30mm x 0.25 mm, coating thickness 0.25 μm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/ min. Helium (99.99%) was the carrier gas at a flow rate of 1 mL/ min. The molecular weight of decomposition products scan 60-200 g/mol range were performed. Diluted samples (1/100 in chloroform, v/v) of 1.0 μL were injected manually. The identification of components was based on the comparison of their mass spectra with those of Wiley 7 N (contains 392.086 compounds spectra), NIST 2002 (contains 174.948 compounds spectra) and flavor (contains 419 compounds spectra) libraries and as well as by comparison of their retention times.

Statistical Analysis

All experiments were done in triplicate. The results were expressed as average ± standard deviations (SD). Statistical analysis was performed on the data by SPSS 15.0 Bivariate Correlation Analysis (SPSS Inc., Chicago). Between ethanol and distilled water extracts were found significant statistically correlation at the 0.01 level.

RESULTS AND DISCUSSION

Determination of Total Antioxidant Status (TAS) of Extracts

Antioxidant components of plants have attracted great interest for the prevention and treatment of complex diseases such as cardiovascular diseases, cancer, diabetes, AIDS, Alzheimer's diseases [14]. Additionally, it was stated in the literature that several types of plant materials such as vegetables, fruits, leaves, seeds, roots and stem barks are the potential sources of antioxidant compounds [15]. The TAS values of *Morus alba* L. leaves for ethanol and distilled water extracts were shown in Table 1. TAS values of ethanol extract of *Morus alba* L. leaves displayed higher capacity than distilled water extract. While TAS values of leaf-ethanol extract was found as 1.56 mmol Trolox Eqv./L, TAS values of leaf- distilled water extract were found as 1.45 mmol Trolox Eqv./L. While the recent report that suggests the antioxidant activity of distilled water extract was better than ethanol extract with DPPH assay, we found better TAS value for ethanol extract with Erel's method [16].

Table 1. TAS levels in *Morus alba* L. leaves extracts

Extracts samples	Total Antioxidant Status*
Ethanol	1,56
Water	1,45

*Total Antioxidant Status were calculated as mmol Trolox Equivalent./L.

Determination of Antimicrobial Activity of Extracts

In this study, it was used two different soluble solutions as ethanol and distilled water. The antimicrobial activity of leaves of *Morus alba* L. is presented in Table 2. The inhibition zones of our test microorganisms were measured as mm and were seen in different rate. The ethanol extracts of leaves were more effective than distilled water extracts. Distilled water extracts have no any inhibition zone. The maximum zone of inhibition was against *Pseudomonas aeroginosa* (13.5±0.5 mm), followed by *Escherichia coli* (9.7±0.9 mm) and *Enterococcus faecalis* (9.5±1.2 mm). The minimum zone of inhibition was obtained against *Candida albicans* and *Staphylococcus aureus*, (8.7±0.9 and 8.7±0.8 mm respectively). It was reported that methanol and chloroform extracts of different species of Morus exhibited antimicrobial activity against *Pseudomonas aeroginosa*, *Proteus vulgaris, Bacillus subtilis, Salmonella typhi, Shigella flexneri*, *Candida albicans* and *Aspergillus niger* [17]. In another study, leaves, stems and fruits of *Morus alba* L. were extracted with ethanol and water. The ethanol extracts exhibited stronger antimicrobial activities than the distilled water extracts. Among the ethanol extracts, leaves had most effective inhibition activity with the IC_{50} 7.11 ± 1.45 mg/ mL value [16]. Our results supported the previous studies.

Table 2. Inhibition rate of herbal extracts from *Morus alba L.* leaves in our test microorganisms

Extracts samples	Strains				
	S.aureus	*E.faecalis*	*P.aeroginosa*	*E.coli*	*C.albicans*
Ethanol	8,7 ± 0.8	9,5 ± 1,2	13,5 ± 0,5	9,7 ± 0,9	8,7 ± 0,9
Water	ND	ND	ND	ND	ND

ND: Not determined

Values are mean of triplicate readings (mean ±S.D).

Gas Chromatography/Mass Spectrometry (GC/ MS)

The extract cleavage products GC/MS spectrum was obtained according to the observed peaks (Figure 1). Structural formulas of the diagram are the appropriate degradation products are displayed on the peaks. The extract components were identified the aid of gas chromatography and decomposition products were characterized by mass analyser detector GC/ MS. The mass spectrum shows that there were three components in extracts, 9,12,15-octadecatrienoic acid ethyl ester, linolenic acid ethyl ester and gibberallic acid respectively, and the giberellic acid is main one. According to Saravanan et al. [18], as a result of GC/ MS analysis of *Ficus religiosa* L. which is same family member with *Morus alba* L. (Moraceae), 13 different compounds were determined. Some of the compounds

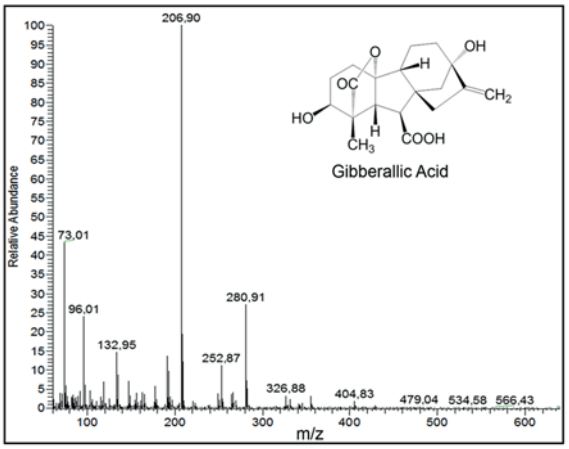

Figure 1. GC/MS spectra and fragmentation patterns of extract

that have antioxidant and antimicrobial activity were phenol, 4-methoxy phenol, ethyl isoallocholate and octadecanoic acid. In another study, GC/MS analysis showed the presence of 9,12,15-octadecatrienoic acid in *Vitis sitosa*. It was reported that this compound possesses many biological activities such as anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, antihistaminic, antieczemic, antiacne, antiandrogenic, antiarthritic, anticoronary, insectifuge properties and acts as an antimicrobial agent [19].

CONCLUSIONS

In this research we enlightened antioxidant and antimicrobial activities with GC/MS analysis of *Morus alba* L. leaves. Our study indicates promising results for the antioxidant and antimicrobial activity of ethanol extracts of *Morus alba* L. leaves. In present study, four different compounds were identified in the ethanol extracts of leaves by GC/MS analysis. Especially, giberellic acid contributed more percentage than other components. But other components such as 9,12,15-octadecatrienoic acid was reported for many biological activities. So, antioxidant and antimicrobial activity can be related to these molecules. Therefore, this research supports of using this plant's leaves for future development of novel antioxidant and antimicrobial agents.

It is also important to emphasize those further biological studies to determine the mechanism of antimicrobial action and to elucidate the other activities of components such as anti-inflammatory, anticancer must be conducted.

ACKNOWLEDGEMENTS

We are thankful to Department of Biology, Gazi University, Prof. Dr. Murat EKICI for identification plants samples.

REFERENCES

1. Salem MZM, Aly H, Gohar Y, El–Sayed AW. Biological activity of extracts from *Morus alba* L., *Albizzia lebbeck* L. Benth. and *Casuarina glauca* Sieber against the growth of some pathogenic bacteria. Journal of Agricultural and Food Chemistry 2 (2013) 9–22.
2. Chang LW, Juang LJ, Wanga BS, Wang MY, Tai HM, Hung WJ, Chen YJ, Huang MH. Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. Food and Chemical Toxicology 49 (2011) 785-790.
3. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects in superoxide radicals. Food Chemistry 64 (1999) 555-559.
4. Harauma A, Murayama T, Ikeyama K, Sano H, Arai H, Takano R, Kita T, Hara S, Kamei K, Yokode M. Mulberry leaf powder prevents atherosclerosis in apolipoprotein E–deficient mice. Biochemical and Biophysical Research Communications 358 (2007) 751-756.
5. Dua J, Hea Z–D, Jianga RW, Yeb WC, Xua HX, But PPH. Antiviral flavonoids from the root bark of *Morus alba* L. Phytochemistry 62 (2003) 1235-1238.
6. Prakash D, Upadhyay G, Sing BN, Sing HB. Antioxidant and free radical scavenging activities of seeds and agri–wast of some varieties of soybean. Food Chemistry 104 (2007) 783.
7. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation more sable ABTS radical cation. Clinical Biochemistry 37(4) (2004) 277–85.
8. Khan MA, Rahman AA, Islam S, Khandokhar P, Parvin S, Islam B, Hossain M, Rashid M, Sadik G, Nasrin S, Mollah MNH, Alam K. A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba* L. (Moraceae). BMC Research Notes (2013) 6–24.
9. Naowaboota J, Pannangpetcha P, Kukongviriyapana V, Kukongviriyapanb U, Nakmareongb S, Itharatc A. Mulberry leaf extract restores arterial pressure in streptozotocin–induced chronic diabetic rats. Nutrition Research 29 (2009) 602-608.
10. Oh J, Jo H, Cho AR, Kim SJ, Han J. Antioxidant and antimicrobial activities of various leafy herbal teas. Food Control 31 (2013) 403–409.
11. Exarchou V, Nenadis N, Tsimidou M. Antioxidant activities and phenolics composition of extracts from Greek oregano, Greek sage and Summer savory. Journal of Agricultural and Food Chemistry 50 (19) (2002) 5294–5299.
12. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. BioMed Research International 2014 (2014) 1–11.

13. Sohn HY, Son KH, Kwon CS, Kwon GS, Kang SS. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica Schneider*, *Broussnetia papyrifera* (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis Nakai. Phytomedicine 11 (2004) 666–672.
14. Muthumani GD, Vijay Anan A, Manikandan A. Antioxidant, antihelmintic and antimicrobial activity of *Euphorbia thymifolia* Linn whole plant. International Journal of Current Microbiology and Applied Sciences 2 (2013) 66.
15. Kahkonen MP, Hopia AI, Vuorela HJ, Rauh JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47 (1999) 3954–3962.
16. Wang W, Zu Y, Fu Y, Efferth T. In vitro antioxidant and antimicrobial activity of extracts from Morus alba L. leaves, stems and fruits. The American Journal of Chinese Medicine 40(2) (2012) 349–356.
17. Aditya Rao SJ, Ramesh CK, Riaz M, Prabhakar BT. Anthelmintic and antimicrobial activities in some species of mulberry. International Journal of Pharmacy and Pharmaceutical Sciences 4 (2012) 335–338.
18. Saravanan P, Chandramohan G, Mariajancyrani J, Shanmugasundaram P. GC–MS analysis of phytochemical constituents in ethanolic bark extract of *Ficus religiosa* LINN. Int. J. Pharm. Sci. 6 (2014) 457–460.
19. Gobalakrishnan R, Manikandan P, Bhuvaneswari R. Antimicrobial potential and bioactive constituents from aerial parts of *Vitis setosa* Wall. Journal of Medicinal Plants Research 8(11) (2014) 454–460.